Effects of root exclusion on nitrogen transformations and decomposition processes in spruce humus

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SUMMARY

1 During March 1983 trenched plots were established in a 37-year old stand of Sitka spruce. Between April and November monthly measurements of exchangeable inorganic nitrogen, rates of nitrogen mineralization, FDA-stained fungal mycelium and humus moisture content were made in rooted and root excluded areas. The ammonium, nitrate and soluble organic nitrogen contents of throughfall and humus leachate, collected using zero tension lysimeters, were also measured.

2 Trenching caused a rapid increase in the rate of nitrogen mineralization and after 8 months trenched areas contained *c.* 21 kg ha^{-1} NH$_4^+$-N, a tenfold increase over rooted areas which remained approximately constant at *c.* 2 kg ha^{-1}. Nitrate was never found in significant quantities.

3 Seasonal trends in FDA-stained fungal mycelium were similar in trenched and untrenched areas with maximum lengths of *c.* 220 m mycelium g^{-1} organic matter occurring in spring and autumn. Length of FDA-stained mycelium positively correlated with humus moisture content.

4 Trenching caused no apparent increase in the rate of organic matter decomposition.

5 The total soluble nitrogen input in throughfall was 9·18 kg ha^{-1} and represented an important source of nitrogen compared to the 11·6 kg ha^{-1} N released by mineralization. Leaching loss from humus in rooted areas was 1·38 kg N ha^{-1}, soluble organic nitrogen being the most important component. Soluble nitrogen leached from trenched areas was approximately 50% of input from throughfall; ammonium was the major constituent.

6 When estimated on an annual basis the forest floor may provide 26·5 kg N ha^{-1}, which is approximately 35% of the estimated uptake requirement for this stand. Reasons for this discrepancy are discussed.

INTRODUCTION

The development of surface organic horizons on freely drained soils beneath coniferous forests is a well documented phenomenon and may play a role in the stabilization of element cycling and nutrient conservation within the ecosystem. However, as a consequence large quantities of nitrogen become unavailable to the

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trees. The resulting growth reduction due to nitrogen stress may be unimportant in stable natural forest ecosystems but in commercial plantations is cause for concern.

A number of factors contribute to the development of these organic layers, not least the poor substrate quality (Swift, Heal & Anderson 1979) of coniferous litter and the immobilization of nitrogen in the soil microbial biomass. However, if their development is indeed related to the closing of nutrient cycles then all components of the ecosystem may be expected to contribute. In 1935 Romell suggested that the high densities of mycorrhizal fine roots and associated mycelium found in surface horizons were a contributory factor. Accelerated decomposition rates of litter in the absence of living fine roots and mycorrhizas have since been demonstrated (Gadgil & Gadgil 1975; Babel 1977; Berg & Lindberg 1980) and attributed to competition for nutrients between mycorrhizal fungi and the saprophytic micro-flora or increased moisture stress in the substrate caused by transpiration.

Trenching (i.e. root exclusion) experiments can shed light on the interaction between roots and soil processes. For example, Romell (1938) attributed the increased lushness of ground vegetation within trenched plots to greater nitrogen availability resulting from reduced competition and green-manuring from dead roots. More recently, Vitousek *et al.* (1982) used trenching to examine the control of nitrogen mineralization and nitrate production in the range of North American forests. In this paper we report the effects of trenching on decomposition rates, fungal activity, and nitrogen mineralization and transformations in the organic horizons of a Sitka spruce (*Picea sitchensis* (Bong.) Carr) stand during the first 8 months following treatment.

METHODS

Study site

In November 1982 three 0·04 ha plots were established in 37-year-old Sitka spruce, yield class 16, growing on an acid brown forest soil of the Countesswells series in Durris forest (National Grid reference NJ733944). There was no understorey and brash was cleared from the plots to facilitate sampling. At six selected sites in each plot a zero-tension lysimeter constructed from $300 \times 92 \times 19$ mm PVC guttering was installed at the junction of the organic horizons and the mineral soil. The depth of the lysimeters varied between 5 and 10 cm below the soil surface. In March 1983 trenches were dug to isolate three irregular 2–3 m^2 areas in each plot, each containing a lysimeter. Soil was excavated to the indurated layer (40–70 cm), all roots severed, and the trenches were backfilled after the inside walls had been lined with a double layer of 800 gauge polythene sheeting.

Sampling

Samples were collected on a monthly basis beginning in April, approximately 2 weeks after trenching. Lysimeter leachate was stored in 1 litre polythene bottles

containing 10 mg $HgCl_2$. Throughfall was collected in similar bottles fitted with a 142 mm diameter polythene funnel plugged with glass wool. Three throughfall bottles were randomized on each plot and rerandomized each month. When rainfall was heavy, intermediate collections were made and saved for analysis with the normal monthly sample. A 1 m^2 permanent quadrat was laid out within each trenched area and three similar quadrats were established in the untrenched parts of each plot. Each month four 40 mm diameter cores were taken in each quadrat to the organic/mineral interface using a collapsible corer. One core was wrapped in plastic film food wrapping and replaced *in situ* for estimation of net nitrogen mineralization and a duplicate core was used for exchangeable mineral nitrogen determination. The six remaining cores from the three trenched and three untrenched quadrats in each plot were bulked, passed through a 5·6 mm sieve and weighed prior to determination of moisture content, fungal activity and loss on ignition.

Analyses

Throughfall and lysimeter leachate was filtered (Whatman No. 44 + 0·22 μm membrane filter) prior to analysis for NH_4^+-N and NO_3^--N. An aliquot of the filtrate was concentrated by boiling and digested with H_2SO_4/H_2O_2 (Allen *et al.* 1974). Soluble organic nitrogen was estimated as the increase in NH_4^+-N in the filtrate following digestion. Exchangeable NH_4^+-N and NO_3^--N in whole soil cores were extracted with 200 cm^3 of 1 M KCl. Extracts were filtered (Whatman No. 42) prior to analyses. Nitrogen determinations were carried out by automated flow colorimetric analysis using Technicon procedures.

Fungal activity in a 5 g subsample of the sieved bulked cores was estimated by counting fluorescein diacetate (FDA)-stained mycelium (Söderström 1977, 1979a). Fluorescing hyphae $\geqslant 1$ μm in diameter were counted. Moisture content was determined by oven drying for 24 hours at 103°C and organic matter by loss on ignition at 450°C for 3 hours.

Analysis of variance showed that exchangeable nitrogen content and rates of mineralization did not differ significantly between the three replicate plots, and data for these have been bulked for graphical representation.

RESULTS

Throughfall and soil moisture content

Between April and November 250 mm of throughfall were collected, distributed as shown in Fig. 1. May and September were the wettest months; August was unusually dry. The moisture content of the organic horizons as estimated from the single monthly sample was closely related to throughfall volume and was lowest in August and November. The effect of root exclusion, and the consequent elimination of
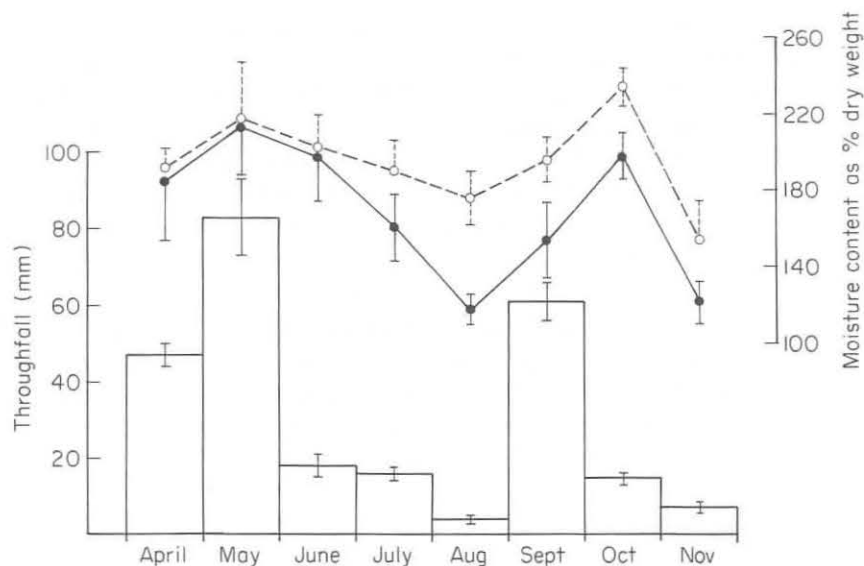
FIG. 1. Throughfall, in mm ± standard errors (open bars) and moisture content as a percentage of dry weight of forest floor (± standard errors) in trenched (○) and untrenched (●) areas between April and November.

evapotranspiration, was apparent between July and October when the moisture content of trenched areas was higher than that of rooted areas.

Nitrogen in throughfall and lysimeter samples

The soluble nitrogen input in throughfall to the forest floor over the study period amounted to $9 \cdot 18$ kg ha^{-1} consisting of $2 \cdot 35$ kg ha^{-1} soluble organic N (26%), $3 \cdot 12$ kg ha^{-1} NH$_4^+$-N (34%) and $3 \cdot 71$ kg ha^{-1} NO$_3^-$-N (40%).The pattern of input was related to throughfall volume (Fig. 2a), although there was some compensatory increase in nitrogen concentration when throughfall volume was low, most obviously during August. The relative proportions of the three forms of soluble nitrogen remained relatively constant throughout the period.

There was considerable variation in leachate output between individual lysimeters. Overall the volume collected from trenched areas ($19 \cdot 9$ litres) was greater than that from untrenched areas ($14 \cdot 5$ litres). Although low volume flow was associated with higher nitrogen concentrations, total output of nitrogen was directly related to the volume discharged by the lysimeters and ultimately to the input from throughfall (Fig. 2b). No lysimeter leachate was collected in August.

The output of nitrogen from lysimeters in rooted areas was $1 \cdot 38$ kg ha^{-1}, 85% less than the input from throughfall, and consisted of $0 \cdot 60$ kg ha^{-1} soluble organic N (43%) $0 \cdot 47$ kg ha^{-1} NH$_4^+$-N (34%) and $0 \cdot 31$ kg ha^{-1} NO$_3^-$-N (23%). The effect of trenching on lysimeter nitrogen output was immediate. There was a 60% increase with 1 month and over the study period $4 \cdot 58$ kg ha^{-1} was discharged, over three
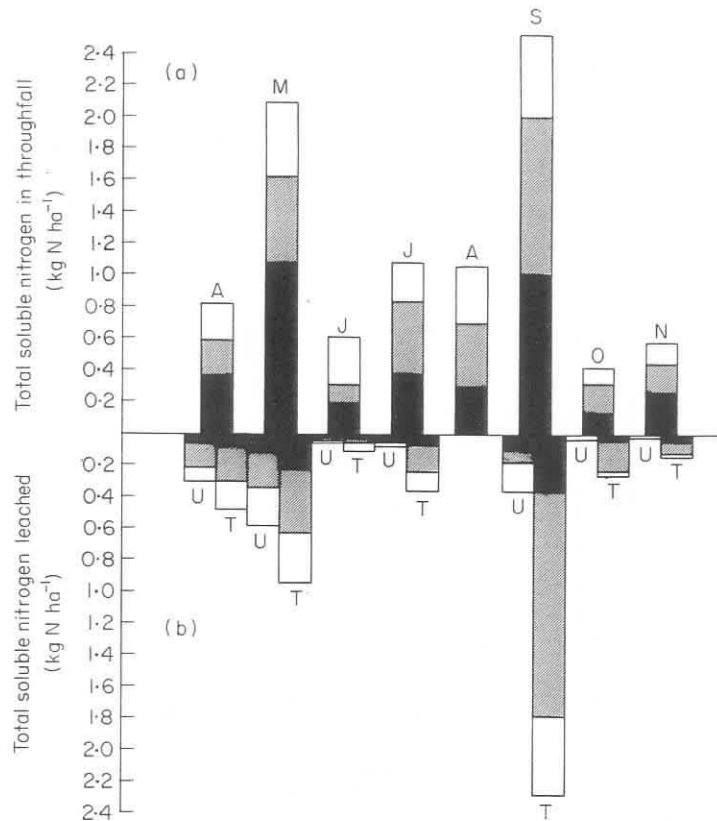
FIG. 2. (a) Total soluble nitrogen input in throughfall (kg N ha^{-1}) between April and November. (b) Total soluble nitrogen (kg N ha^{-1}) leached from untrenched (U) and trenched (T) areas between April and November. Where bars are compartmentalized components of total kg N ha^{-1} are NO$_3^-$-N (solid), NH$_4^+$-N (hatched) and soluble organic nitrogen (open).

times that from untrenched lysimeters, but still only 50% of the throughfall input. The output was made up of 1·20 kg ha^{-1} soluble organic N (26%), 2·25 kg ha^{-1} NH$_4^+$-N (56%) and 0·82 kg ha^{-1} NO$_3^-$-N (18%), representing an increase in the proportion of NH$_4^+$-N after trenching. Almost 50% of the output followed heavy rains during September.

Exchangeable nitrogen and net nitrogen mineralization

The amount of exchangeable NH$_4^+$-N in the organic horizons outside the trenches, c. 2 kg ha^{-1}, did not change significantly throughout the study period (Fig. 3). Exchangeable NO$_3^-$-N was always less than 0·1 kg ha^{-1}. Trenching had an immediate effect on exchangeable NH$_4^+$-N which rose to c. 4 kg ha^{-1} within 2 months and after June rose more rapidly to a maximum of 21·3 kg ha^{-1} by November. This upward trend was interrupted in October, the temporary drop at that time being
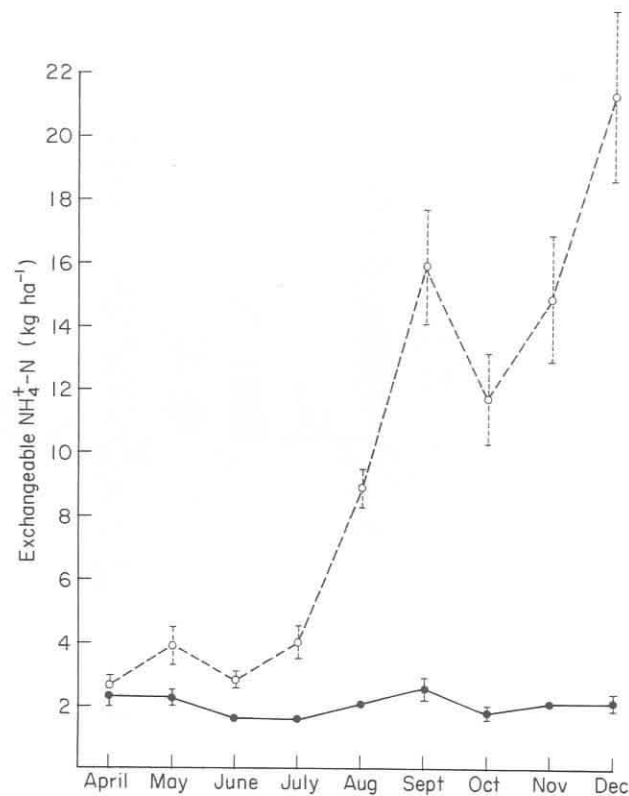
FIG. 3. Quantities of exchangeable NH_4^+-N (kg ha^{-1}) in trenched (○) and untrenched (●) areas between April and December. When not shown standard errors are contained within the data point.

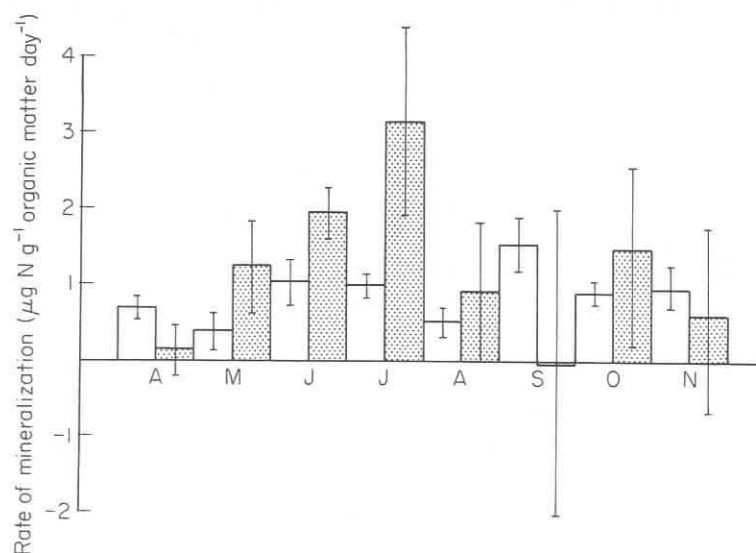FIG. 4. Rate of mineralization (μg N g^{-1} organic matter day^{-1}, $\pm$ standard errors) in trenched (stippled) and untrenched (open) areas between April and November.

reflected in the wash-out of NH_4^+-N in the lysimeter samples during September. Despite this large accumulation of NH_4^+-N, levels of NO_3^--N remained low after trenching and had reached only 0·3 kg ha^{-1} by November.

There was no significant increase in the amount of exchangeable NO_3^--N during incubation of cores either from inside or outside the trenches. In the absence of nitrification net mineralization is therefore equivalent to the increase in the level of NH_4^+-N. Outside the trenches the mean monthly rate ranged from 0·39 to 1·53 μg N g^{-1} organic matter day^{-1}. The rate was lowest in May and August and highest in September (Fig. 4). The rate of mineralization within the trenches rose steadily to a peak of 3·16 μg N g^{-1} during July. Thereafter it fell, there was increased variation between cores, and in a significant number net immobilization of nitrogen occurred. No relationship could be found between mineralization rates and the forest floor moisture content value for the month during which incubation took place.

The total net nitrogen mineralization over the study period amounted to 11·6 kg ha^{-1} outside, and 17·5 kg ha^{-1} inside, the trenches.

FDA-stained hyphae

The mean length of FDA-stained mycelium in the samples ranged from 130 to 230 m g^{-1} organic matter (Fig. 5). Although the variation was considerable samples from outside the trenched areas showed distinct peaks of activity in May and
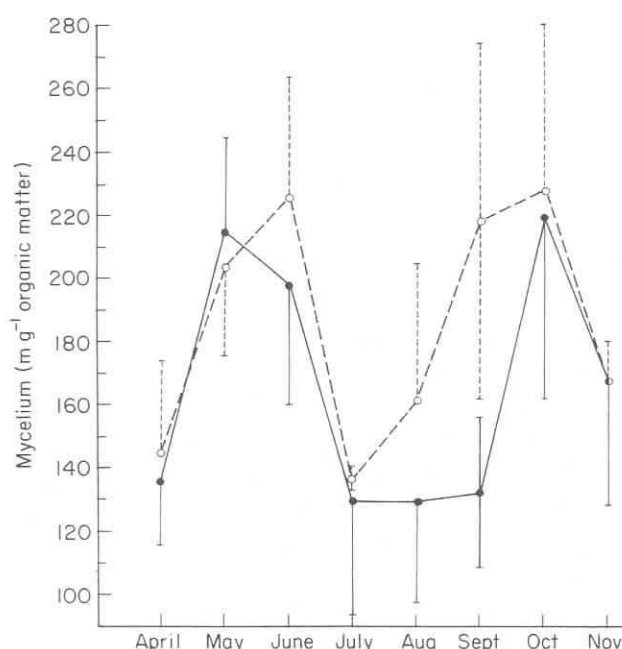

FIG. 5. Mean length of FDA-stained mycelium (m g^{-1} organic matter, ± standard errors) for trenched (○) and untrenched (●) areas between April and November.
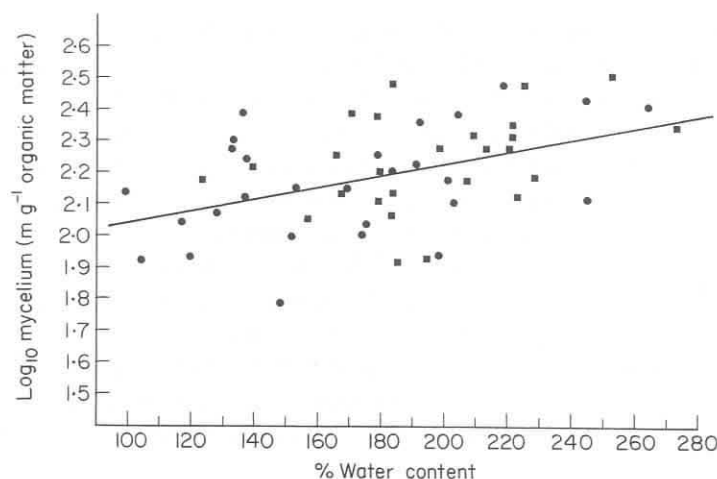
FIG. 6. Relation of total length of FDA-stained mycelium ($\log_{10}$ metres g^{-1} organic matter) to percentage water content of forest floor, (trenched areas, ■; untrenched areas, ●). $Y = 0.001x + 1.85$; $r = 0.45$; ($P = 0.001$).

October and a trough from July to September. Samples from within the trenches followed the same pattern except during August and September when activity rose sharply. At this time the reduced moisture content outside the trenches was most marked (Fig. 1) and Fig. 6 shows that there was indeed a significant relationship between moisture content and the length of FDA-stained mycelium over the study period.

Organic matter accumulation

The mean weight of organic matter on the forest floor in April was 59 000 ± 8800 kg ha^{-1}. There was no significant change over the course of the experiment or in response to trenching.

DISCUSSION

The current study has shown that the trenching has a substantial effect on both the amount of inorganic nitrogen and rate of mineralization within the forest floor. Whilst this may be due to the exclusion of functioning roots, trenching may have other confounding effects. Reduction of water loss by the elimination of transpiration elevated the humus water content of trenched plots to levels often greater than those of rooted areas (Fig. 1). Although this may not have a significant effect on suppression of litter decomposition (Gadgil & Gagdil 1975) water availability is known to influence rates of nitrogen mineralization in mineral soils (Myers, Campbell & Weier, 1982; Matson & Vitousek 1981; Miller & Johnson 1964) and humus. Laboratory incubations of Scots pine humus showed a 6–7 fold reduction in its mineralization rate when humus water content was reduced to 80% of its maximum

(Clarholm *et al.* 1981). The minimum moisture content of rooted areas occurred during August and was about 20% less than the maximum recorded in October, thus mineralization may have been affected. However, there was no correlation between moisture content and either exchangeable nitrogen or rates of mineralization; moreover effects of trenching were immediate, and occurred when trenched and rooted areas had similar water contents (Figs 1 and 4).

Although roots severed during trenching may act as a green manure and enhance the availability of nitrogen (Romell 1938) this may be of little significance in Sitka humus. The mean standing crop of fine roots and mycorrhizas less than 5 mm in diameter for 35-year old Sitka spruce is about 1000 kg ha^{-1} (Alexander & Fairley 1983). Assuming these to contain 1·5% nitrogen, trenching would provide *c.* 15 kg N ha^{-1}. These amounts are very small when compared to the quantity of *c.* 1000 kg N ha^{-1} contained in a forest floor with a dry weight of *c.* 59 000 kg ha^{-1} organic matter.

Moisture did appear to account for the slight difference in FDA-active hyphal lengths attributable to trenching. In general, lengths followed a similar seasonal pattern to that found elsewhere (Söderström 1979b) irrespective of trenching, although the values (250 m g^{-1}) are low compared to other studies. In August and September it seemed that activity in rooted areas was depressed by low soil moisture content (Figs 1 and 5). However, a number of other unknown factors also influence hyphal activity. It might be expected that trenching would reduce hyphal lengths as mycorrhizal fungi decline but the persistence of excised roots over the study period to date lessens that possibility (Ferrier & Alexander, p. 175).

The forest floor contains a large amount of organic nitrogen, decomposition of which is generally assumed to be an important source for tree growth. Nitrogen mineralization in the trenched areas between April and November was estimated to be *c.* 17·5 kg N ha^{-1} an increase of approximately 35% over rooted areas for the same period. On a yearly basis rooted areas mineralized *c.* 14·3 kg N ha^{-1}, which was very low when compared with the 67 kg N ha^{-1} reported for Sitka spruce litter and humus by Williams (1983). This large discrepancy may result from differences in experimental methodology. Whereas Williams measured accumulation by sub-sampling sieved litter within enclosed pots in the field, the current study utilized intact cores isolated at monthly intervals.

There was no evidence of significant NO_3^--N in soil extracts, soil incubations or lysimeter samples. Vitousek *et al.* (1982) have discussed the processes which might account for such a situation. In view of the ready availability of NH_4^+-N, particularly in trenched areas, competition for NH_4^+-N cannot be the cause. Rapid denitrification seems equally unlikely. Delayed or inhibited nitrification due to allelochemic suppression of nitrifiers, competition for some other limiting nutrient, or low initial populations of nitrifiers are more probable. In the latter circumstance an increase in NO_3^--N might be expected in trenched plots as the study proceeds.

Input of nitrogen in throughfall was relatively high when compared to other coniferous systems. When estimated on annual basis the recorded quantities of 3·71 kg NO_3^--N ha^{-1} and 3·12 kg NH_4^+-N ha^{-1} were above the median values for

the ranges $0.22-20.00$ kg NO_3^--N and $0.2-6.4$ kg NH_4^+-N given by Parker (1983). The total soluble nitrogen input of 9.18 kg ha^{-1} was similarly high and when compared to the 11.6 kg ha^{-1} N mineralized within the forest floor, represents an important source of nitrogen. Input in throughfall was about six times greater than leaching from rooted areas suggesting utilization or retention of nitrogen within the forest floor. Exclusion of roots increased loss by leaching to 50% of input and was mainly due to the raised concentrations of NH_4^+-N in the leachate. This is frequently observed in trenched areas (Vitousek *et al.* 1982).

The sum of inorganic nitrogen input in throughfall and that estimated to be mineralized in trenched areas amounted to 24.33 kg ha^{-1}. Allowance for leaching loss of 3.37 kg ha^{-1} gives a theoretical increase of 20.96 kg ha^{-1} of exchangeable nitrogen which compares with a measured increase of 18.7 kg ha^{-1} ($21.3-2.6$, Fig. 3). As quantities of exchangeable inorganic nitrogen within rooted areas changed little between April and November, the figure of 17.65 kg ha^{-1} derived by similar calculation represents the amount of nitrogen available for uptake by roots. Estimated over 1 year the forest floor may provide 26.5 kg N ha^{-1}. This represents about 50% of the average annual uptake requirement of coniferous trees (Cole & Rapp 1981) or 35% of an estimated requirement of 75 kg N ha^{-1} for yield class 16 Sitka spruce (H. G. Miller, pers. comm.). This shortfall may be made good from sources in the mineral soil. Alternatively this large discrepancy between input and uptake requirement may be caused by immobilization of nitrogen by death of fine roots within the incubation (Popović 1980). However, excised fine roots may remain alive for a considerable time, and fine roots can be found several years after clear felling Scots pine (Persson 1982). Mycorrhizal fine roots of Sitka spruce may survive 8 months in litter bags and FDA-stained mycorrhizal sheath tissue was found in root excluded areas several months after trenching (Ferrier & Alexander, p. 175). There is a strong possibility that during short-term incubations, particularly when using undisturbed cores, mineralized nitrogen is absorbed by living mycorrhizal roots. The greatest rate of mineralization measured for rooted areas occurred during September (Fig. 4), when the mean number of live root tips within the incubating cores was at a minimum (R. Harmer & I. J. Alexander, unpubl.).

On our study site over an 8 month period, trenching did not appear to increase rates of organic matter decomposition as suggested by Gadgil & Gadgil (1975) or Berg & Lindberg (1980). Moreover, as readily extractable NH_4^+-N accumulated in trenched areas it is unlikely that in our system microbial growth or breakdown processes are limited by nitrogen availability and therefore not surprising that removal of root competition had no effect. This would also explain the similarity in FDA-stained hyphal lengths inside and outside trenched areas. It seems much more likely that microbial activity and organic matter decomposition are governed by the intractable nature of Sitka spruce litter as a carbon source. The low FDA-stained hyphal lengths recorded here support this conclusion.

ACKNOWLEDGMENTS

REFERENCES

Alexander, I.J. & Fairley, R.I. (1983). Effects of N fertilisation on populations of fine roots and mycorrhizas in spruce humus. *Plant and Soil*, **71**, 49–53.

Allen, S.E., Grimshaw, H.M., Parkinson, J.A. & Quarmby, J. (1974). *Chemical Analysis of Ecological Material*. Blackwell Scientific Publications, Oxford.

Babel, U. (1977). Influence of high densities of fine roots of Norway spruce on processes in humus covers. *Soil Organisms as Components of Ecosystems* (Ed. by U. Lohm and T. Persson), *Ecological Bulletins (Stockholm)*, **25**, 584–586.

Berg, B. & Lindberg, T. (1980). Is litter decomposition retarded in the presence of mycorrhizal in forest soil? *Swedish Coniferous Forest Project Internal Report*, **95**, 10 pp.

Clarholm, M., Popović, B., Rosswall, T., Söderström, B., Sohlenius, B., Staff, H. & Wiren, A. (1981). Biological aspects of nitrogen mineralisation in humus from a pine forest podsol incubated under different moisture and temperature conditions. *Oikos*, **37**, 137–145.

Cole, D.W. & Rapp, M. (1981). Elemental cycling in forest ecosystems. *Dynamic Properties of Forest Ecosystems* (Ed. by D.E. Reichle), pp. 341–409. Cambridge University Press, Cambridge.

Gadgil, R.L. & Gadgil, P.D. (1975). Suppression of litter decomposition by mycorrhizal roots of *Pinus radiata*. *New Zealand Journal of Forestry Science*, **5**, 33–41.

Matson, P.A. & Vitousek, P.M. (1981). Nitrogen mineralisation and nitrification potentials following clearcutting in the Hoosier National Forest, Indiana. *Forest Science*, **27**, 781–791.

Miller, R.D. & Johnson, D.D. (1964). The effect of soil moisture tension on carbon dioxide evolution, nitrification and nitrogen mineralisation. *Soil Science Society of America Proceedings*, **28**, 644–647.

Myers, R.J.K., Campbell, C.A. & Weier, K.L. (1982). Quantitative relationship between net nitrogen mineralisation and moisture content of soils. *Canadian Journal of Soil Science*, **62**, 111–124.

Parker, G.G. (1983). Throughfall and stemflow in the forest nutrient cycle. *Advances in Ecological Research*, **13**, 1–134.

Persson, H. (1982). Changes in the tree and dwarf shrub fine-roots after clear cutting in a mature Scots pine stand. *Swedish Coniferous Forest Project Technical Report*, **31**, 19 pp.

Popović, B. (1980). Mineralisation of nitrogen in incubated soil samples from an old Scots pine forest. *Structure and Function of Northern Coniferous Forests–An Ecosystem Study* (Ed. by T. Persson). *Ecological Bulletins (Stockholm)*, **32**, 411–418.

Romell, L.G. (1935). Ecological problems of the humus layer in the forest. *Cornell University Agricultural Experimental Station Memoir* No. 170.

Romell, L.G. (1938). A trenching experiment in spruce forest and its bearing on problems of mycotrophy. *Svensk Botanisker Tidskrift*, **32**, 89–99.

Söderström, B.E. (1977). Vital staining of fungi in pure cultures and in soil with fluorescein diacetate. *Soil Biology and Biochemistry*, **9**, 59–63.

Söderström, B.E. (1979a). Some problems in assessing the fluorescein diacetate active fungal biomass in the soil. *Soil Biology and Biochemistry*, **11**, 147–148.

Söderström, B.E. (1979b). Seasonal fluctuations of active fungal biomass in horizons of a podzolised pine-forest soil in central Sweden. *Soil Biology and Biochemistry*, **11**, 149–154.

Swift, M.J., Heal, O.W. & Anderson, J.M. (1979). *Decomposition in Terrestrial Ecosystems*. Blackwell Scientific Publications, Oxford.

Vitousek, P.M., Gosz, J.R., Grier, C.C., Melillo, J.M. & Reiners, W.A. (1982). A comparative analysis of potential nitrification and nitrate mobility in forest ecosystems. *Ecological Monographs*, **52**, 155–177.

Williams, B.L. (1983). Nitrogen transformations and decomposition in litter and humus from beneath closed-canopy Sitka spruce. *Forestry*, **56**, 17–32.